NOTE

Pituitary Luteinizing Hormone Level in the Male House Musk Shrew, *Suncus murinus* L., Following Castration and Testosterone Treatment

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Abstract

The effect of castration and replacement therapy with testosterone propionate (TP) on the pituitary LH concentration and contents in the house musk shrew was investigated by using an *in vitro* bioassay for LH, the Rat Interstitial Cell Testosterone assay. The concentration and contents of LH increased slightly 10 days after castration, but decreased progressively thereafter to about a half of the pre-operation level by 90 days after the operation. The replacement with TP (100 µg/day) for 7 days significantly depressed LH contents when it was begun 10 days after castration, while the same treatment started immediately after or 30 days after the operation did not significantly affect the pituitary LH level. The feedback mechanism between the gonad and the pituitary may be slightly different in the shrew from that in other mammals.

TP replacement, started immediately after castration, completely inhibited the decrease in the weight of male accessory sex organs in castrated shrews. In castrated animals when more than 10 days had elapsed after the operation, however, the decreased weight of the organs could not be fully restored by the TP replacement for 7 days.

During the last few years we have investigated various reproductive traits in the house musk shrew, *Suncus murinus* L., a new laboratory animal of the insectivore (Furumura *et al.*, 1982, 1983, 1984). Although the concentration and contents of the steroid hormone either in the plasma or the tissues of the house musk shrew have been reported (Hasler *et al.*, 1975; Hasler and Nalbandov, 1978; Furumura *et al.*, 1983, 1984), information on the level of the peptide

hormones in this species have not yet been available. Since no cross reaction was found between the pituitary homogenates of the house musk shrew and the presently available anti-serum for luteinizing hormone (LH) (Wakabayashi, personal communication), a specific bioassay (Rat Interstitial Cell Testosterone assay, RICT assay; Dufau *et al.*, 1974, 1976) was applied for the measurement of pituitary LH. The present study aimed at providing basic information on the pituitary LH level in the house musk shrew by elucidating the effects of castration and

testosterone replacement on the level.

In the female shrew, ovariectomy or estrogen treatment affects neither uterine and vaginal histology nor sexual receptivity (Dryden and Anderson, 1977). Therefore, the effect of castration and testosterone replacement on the weight of the male accessory sex organs was also studied.

Materials and Methods

Animals

The house musk shrew, brought from southern Japan (Furumura *et al.*, 1983), has been maintained as a closed colony for over 10 generations. Adult male animals weighing 53–80 g (5 to 20 months old) were used in the present study. They were housed individually in polycarbonate cages (18×25×13 cm) and kept in a temperature (20–30°C)—and light (14L:10D, lights on 0500 h)—controlled animal room. The commercial food pellets for young rainbow trout (3P: Chubu Shiryō Co., Ltd, Nagoya, Japan) and water were given *ad libitum*. A small amount of boiled egg or canned bonito flakes (CK: Oriental Kobo Kogyo K.K., Tokyo, Japan) was also given once a week.

Male rats (Wistar-Imamichi strain, Dobutsu Hanshoku Kenkyujo, Omiya, Japan) weighing 150–250 g were used as the donors of the testicular cells for the RICT assay.

Orchidectomy and testosterone replacement in the house musk shrew

The testes were removed under light ether anesthesia and the castrated animals were killed 10 (♂ 10 group), 30 (♂ 30 group) or 90 (♂ 90 group) days after the operation. Adult male shrews over 9 months old served as the intact control (Intact group).

For the replacement experiment, another series of castrated animals were injected with 100 µg of testosterone propionate (TP) in 0.1 ml soybean oil or the same amount of vehicle s.c. daily for 7 days from the day of operation (0-TP, -Oil groups) or from 10 (10-TP, -Oil groups) or 30 days (30-TP, -Oil groups) after the operation. On the morning following the last injection, i.e. 8, 18 and 38 days after castration, the animals

were bled to death under ether anesthesia by heart puncture. Pituitary, adrenal and musk glands were removed and weighed. The pituitary gland was stored at –20°C until used for LH assay. After the removal of the adrenal gland, the caudal half of the body trunk was fixed with 10% formalin for 18 to 20 hrs and then seminal vesicles and ventral prostate glands were dissected out and weighed. Blood was centrifuged at 3,000 rpm for 15 min at 4°C and plasma was stored at –20°C for the testosterone measurement.

Measurement of pituitary LH by RICT assay

For each assay, testes from 2 male rats were used. Interstitial cells were isolated and prepared for RICT assay according to the method of Dufau *et al.* (1974, 1976). The cells from 4 testes were pooled and suspended in 10 ml of Medium 199 containing 1% bovine serum albumin (M199-BSA; pH 7.4), and incubated in a sealed glass vial for 60 min at 37°C under 95% O₂–5% CO₂ gas phase with continuous shaking at 80 strokes/min. After preincubation, the medium was renewed and viability of the cells were checked by trypan blue staining. Finally, the cells were suspended in M199-BSA at a density of 2.0×10⁶ cells/ml and used for the assay. Pituitaries from 2 shrews were pooled and assayed at one time. The glands stored at –20°C were thawed, homogenized with M199 using a teflon pestle homogenizer under ice-cold conditions. The homogenate was let stand in ice for 30 min and then centrifuged at 3,000 rpm for 20 min at 4°C. The supernatant obtained was diluted with M199-BSA to the appropriate concentrations in each assay (125–500 µg eq. of pituitary/ml). Ovine LH was dissolved in M199-BSA. Five dosage levels (3.75–60.0 ng/ml) were prepared to make the standard curve.

The interstitial cells with the pituitary extract or the hormone was incubated at 37°C for 3 hrs under 95% O₂–5% CO₂ gas phase by using glass tubes (15×105 mm). Each tube contained 0.5 ml of the rat interstitial cell suspension and 0.5 ml of the pituitary extract or LH solution. Tubes containing 0.5 ml of the buffer medium, instead of the extract or the hormone solution, were prepared as the incubation control (Control). All assays were performed in quadruplicate. The incubation was ended by chilling the glass tubes in ice-cold water for 5 min. The cell free supernatant was obtained by centrifuge at 1,000×G for 10 min at 4°C and stored –20°C until RIA for testosterone.

Radioimmunoassay for testosterone

Testosterone was measured by RIA using the rabbit antiserum to testosterone-11 α -succ-BSA. The incubation medium was used for the assay directly or after being diluted with 0.01M phosphate buffer saline (pH 7.0) at an appropriate rate (up to 30 times). The plasma testosterone was extracted by ether and then assayed.

Hormones and other drugs

Ovine LH (NIAMDD-LH-S-21) was supplied by the Hormone Distribution Committee, National Institute of Arthritis, Metabolic and Digestive Diseases (Bethesda, Maryland, USA). Bovine albumin was obtained from Reheis Chemical Co. (Phoenix, Arizona, USA). The rabbit antisera to testosterone-11 α -succ-BSA and [1, 2,

6, 7, -³H] testosterone (98.8 Ci/m mol) were purchased from Teikoku Hormone Mfg. Co. (Kawasaki, Japan) and New England Nuclear (Boston, Massachusetts, USA), respectively.

Calculations

The range of doses giving a linear dose response curve for the oLH standard and samples, and the parallelism between curves for the standard and samples were determined by the method of Bliss (1952). The LH potency in samples was obtained by using 2 doses each of the standard and the sample according to the method of Borth (1960) for multiple design analysis. Duncan's multiple range test (Duncan, 1955) was used for the comparison of group means.

Fig. 1. Dose response curves of testosterone production in response to pituitary extracts of the house musk shrew and to oLH by the testicular interstitial cells of the rat cultured *in vitro*. The response is expressed in terms of amounts of testosterone released (ng/assay tube) during incubation for 3 hrs with 1.0×10^6 cells/tube. ▲: Pituitary extract of the shrew 30 days after castration, ■: Pituitary extract of the shrew received testosterone propionate (100 μ g/0.1 ml of soybean oil/day) for 7 days from 30 days after castration, ●: oLH. Each point with vertical bars represents the mean $\pm$ S.E.M. of quadruplicate estimations.

Results

Application of RICT assay for the determination of pituitary LH of the house musk shrew

A standard curve for oLH was obtained over the range of doses from 3.75 to 60.0 ng/ml of M199-BSA. An eminent increase in testosterone production of the rat interstitial cell incubated *in vitro* was caused by adding the pituitary extract of the house musk shrew to the medium. The dose-response curves were linear over the range from 125 to 500 μ g eq. of pituitary/ml, roughly corresponding to 1/8 and 1/2 pituitary/ml, and no significant deviations from parallelism between the dose response curves obtained for oLH and the pituitary samples were observed in any assays. Typical samples are shown in Fig. 1, in which the regression equations for oLH and pituitary extracts of the castrated animal with or without TP replacement were $Y=2.26 \log X - 0.77$ ($\lambda=0.11$), $Y=2.78 \log X - 4.16$ ($\lambda=0.09$) and $Y=2.33 \log X - 2.83$ ($\lambda=0.10$), re-

spectively, where Y is the amount of testosterone released per tube during the 3 hr incubation period, X is the dose of ovine LH (3.75–60.0 ng/ml) or the pituitary extract (125–500 μ g eq. of pituitary/ml) and λ is the precision index of the dose-response line.

Effects of orchidectomy and testosterone replacement on the pituitary LH level in the shrew

The change in the concentration of pituitary LH with time after castration was very similar to that in the contents (Table 1). The concentration and contents had increased slightly by 10 days postcastration, though no significant difference in the values was obtained between the castrated and the intact control. A significant decrease in the concentrations and contents was observed at 30 and 90 days postcastration. The amounts of LH contained in the pituitary of $\oslash 30$ and $\oslash 90$ were about 55% and 45% of those of the intact animals and 50% and 44% of those of the $\oslash 10$, respectively.

Table 1. Effects of orchidectomy and testosterone replacement on the pituitary LH level in the house musk shrew. Castrated shrews were injected with testosterone propionate (100 μ g/0.1 ml soybean oil/day) or vehicle (0.1 ml soybean oil/day) for 7 days from 0, 10 or 30 days postoperation.

Groups	Days after castration		Pituitary LH	
	Start of injection	Autopsy	Concentration (ng/mg)	Content (ng/gland)
Intact	—	—	58.13 $\pm$ 3.36 ^{a,1)}	88.25 $\pm$ 3.76 ^{ab}
Castration				
$\oslash 10$	—	10	65.45 $\pm$ 6.41 ^a	98.18 $\pm$ 4.86 ^a
$\oslash 30$	—	30	31.83 $\pm$ 5.97 ^{cd}	50.68 $\pm$ 5.77 ^{de}
$\oslash 90$	—	90	26.15 $\pm$ 1.04 ^d	42.78 $\pm$ 4.06 ^e
TP-replacement				
0-Oil	0	8	56.70 $\pm$ 3.35 ^{ab}	82.35 $\pm$ 6.54 ^{abc}
0-TP	0	8	59.15 $\pm$ 3.40 ^a	89.53 $\pm$ 12.00 ^{ab}
10-Oil	10	18	56.73 $\pm$ 4.51 ^{ab}	93.13 $\pm$ 7.81 ^a
10-TP	10	18	44.10 $\pm$ 5.78 ^{bc}	65.48 $\pm$ 6.85 ^{cd}
30-Oil	30	38	31.80 $\pm$ 1.38 ^{cd}	58.08 $\pm$ 1.73 ^{de}
30-TP	30	38	34.35 $\pm$ 2.63 ^{cd}	69.20 $\pm$ 9.60 ^{bcd}

1) Mean $\pm$ S.E.M. of 4 determinations in each of which pituitaries of 2 animals were pooled.

a—d: In Duncan's test no significant differences ($P>0.05$) were found between the values having the same superscript in each column.

Table 2. Effects of orchidectomy and testosterone replacement on the organ weights and plasma testosterone concentrations in male house musk shrews (Mean $\pm$ S.E.M. N=8).

Groups	Autopsy, Days after castration	Seminal vesicle ¹⁾ (mg)	Ventral prostate gland (mg)	Pituitary gland (mg)	Adrenal gland ¹⁾ (mg)	Musk gland ¹⁾ (mg)	Plasma testosterone conc. (ng/ml)
Intact	—	96.40 $\pm$ 6.14 ^a	99.90 $\pm$ 6.69 ^b	1.53 $\pm$ 0.08 ^a	9.51 $\pm$ 0.58 ^a	549.60 $\pm$ 20.84 ^{a,2)}	1.57 $\pm$ 0.29 ^b
Castration							
♂ 10	10	42.98 $\pm$ 1.74 ^c	41.34 $\pm$ 2.90 ^d	1.53 $\pm$ 0.12 ^a	10.39 $\pm$ 0.67 ^a	453.83 $\pm$ 13.99 ^a	0.24 $\pm$ 0.05 ^c
♂ 30	30	27.91 $\pm$ 3.13 ^d	27.35 $\pm$ 2.95 ^{ef}	1.71 $\pm$ 0.22 ^a	10.00 $\pm$ 0.55 ^a	383.23 $\pm$ 29.85 ^{a,2)}	0.21 $\pm$ 0.04 ^c
♂ 90	90	21.30 $\pm$ 2.65 ^d	21.71 $\pm$ 2.85 ^f	1.64 $\pm$ 0.16 ^a	10.55 $\pm$ 1.01 ^a	—	0.24 $\pm$ 0.04 ^c
TP-replacement							
0-Oil	8	71.38 $\pm$ 8.32 ^b	55.10 $\pm$ 4.44 ^c	1.45 $\pm$ 0.07 ^a	10.06 $\pm$ 0.52 ^a	374.40 $\pm$ 24.05 ^a	0.20 $\pm$ 0.04 ^c
0-TP	8	96.81 $\pm$ 6.92 ^a	114.45 $\pm$ 8.29 ^a	1.50 $\pm$ 0.11 ^a	9.73 $\pm$ 0.31 ^a	509.10 $\pm$ 42.97 ^a	2.94 $\pm$ 0.29 ^a
10-Oil	18	29.41 $\pm$ 1.69 ^{ed}	31.08 $\pm$ 1.78 ^{def}	1.65 $\pm$ 0.10 ^a	9.41 $\pm$ 0.25 ^a	271.83 $\pm$ 8.27 ^a	0.15 $\pm$ 0.03 ^c
10-TP	18	64.98 $\pm$ 3.82 ^d	61.91 $\pm$ 4.44 ^c	1.50 $\pm$ 0.07 ^a	10.09 $\pm$ 0.24 ^a	479.91 $\pm$ 9.94 ^a	2.63 $\pm$ 0.24 ^a
30-Oil	38	19.06 $\pm$ 1.68 ^d	20.49 $\pm$ 2.46 ^f	1.84 $\pm$ 0.11 ^a	9.81 $\pm$ 0.56 ^a	346.41 $\pm$ 23.42 ^a	0.16 $\pm$ 0.03 ^c
30-TP	38	42.55 $\pm$ 6.63 ^c	37.33 $\pm$ 3.64 ^{de}	2.00 $\pm$ 0.15 ^a	10.48 $\pm$ 0.62 ^a	434.38 $\pm$ 27.13 ^a	2.90 $\pm$ 0.21 ^a

1) Bilateral glands were weighed together.

2) Data for 4 animals.

a—f: In Duncan's test no significant differences (1% level) were found between the values having the same superscript in each column.

There were no significant differences in LH concentrations and contents among the intact, 0-TP and 0-Oil groups. TP administered from 10 days postcastration resulted in a significant decrease in the contents compared with that in the 10-Oil or 10 groups. On the other hand, a slight increase, though not significant, was induced in the concentrations and contents compared with the 30-Oil or 30 groups (Table 1) by the administration of TP from 30 days postcastration.

Effects of orchidectomy and testosterone replacement on the weight of male accessory sex organs and plasma testosterone concentration

Castration resulted in a significant decrease of the weight of male accessory sex organs (Table 2). Weights of both the seminal vesicle and the prostate gland at 10, 30 and 90 days postcastration were 45%, 30% and 20% of the intact males, respectively. Plasma T concentration was depleted to about 15% of the level in the intact by 10 days after castration and this level was maintained thereafter.

Treatment with 100 µg/day of TP for 7 days started from the day of operation effectively maintained the weight of the seminal vesicle and significantly increased the weight of the prostate gland compared with the weight in the intact group. Significant differences ($P < 0.01$) between the weight of both organs in the TP treated animals and the vehicle injected control were obtained at 8 days after castration. Although TP administration from 10 or 30 days postcastration failed to restore the weight of the accessory sex organs to that of the intact males, the weight of the organs was definitely increased by the administration, compared with those of the corresponding controls. Plasma testosterone levels in the TP treated animals were about twice as high as those in the intact animals.

No significant differences were found in the weights of the pituitary and the adrenal

gland among all experimental groups. The weight of the musk gland decreased after castration, though the pattern of change after 10 days of postcastration was not so obvious as that in the accessory sex organs.

Discussion

The RICT assay of Dufau *et al.*, (1974, 1976) has been widely accepted as a reliable specific assay for LH. In parallel with the present series of experiments, we also proved that the addition of FSH or PRL, alone or together with LH, into the incubation medium neither interfered nor augmented the *in vitro* testosterone production of the rat interstitial cells (Furumura *et al.*, unpublished data). The assay has been used for the determination of LH not only in the pituitary gland of the human (Dufau *et al.*, 1976), but also in the blood of the human (Dufau *et al.*, 1976) and the monkey (Dufau *et al.*, 1977). The present study clearly revealed that the assay could be successfully used for the measurement of LH in the pituitary of the shrew as well as the human, although the RICT assay was not applied for the determination of plasma LH in the house musk shrew.

The content and concentrations of the shrew pituitary LH increased slightly 10 days after castration. The increase, however, was only temporary and the pituitary level of LH decreased to about a half of that before the operation during the following 20 days. This pattern of change is quite different from that observed in the castrated rat, in which pituitary LH once decreased around 5 days postoperation, and increased rapidly until 2 weeks postcastration and then gradually thereafter (Badger *et al.*, 1978; Clayton, 1981). If we assume that a negative feedback relationship between LH and testosterone secretions also exists in the house musk shrew as in other species, e.g. in the rat (Damassa *et al.*, 1976) and the monkey

(Plant, 1982), the results of the present study could be explained as follows: The slight increase in the LH content obtained 10 days after castration was due to an enhanced production of the hormone caused by the relief from the negative feedback of androgens. On the other hand, in the animals which spent a longer period after the operation, acceleration of the release of LH exceeded the rate of increase in the synthesis and then the amount of the hormone stored in the pituitary decreased. Replacement with TP strongly suppressed the production of LH in the initial stage of post-operation period, but had an even more profound effect on the release of the hormone in the later stage. This explanation is based on the assumptions that the balance of production and release of LH changes with time after castration and that the suppressing effects of testosterone on the production and the release vary in different states of pituitary function. It was demonstrated in the rat that the inhibitory effect of androgen on LH release was exerted at both the hypothalamic and pituitary levels (Ferland *et al.*, 1976). Divergent effects of castration or TP replacement on the production and the release of LH at different stages of postcastration period would be probable.

Another plausible interpretation of the present result is the change in the number of LH producing cells in the pituitary of chronically castrated shrews. In the rabbit, Foster and Cameron (1964) observed histologically that gonadectomy induced a hyperplasia of the gonadotropic cells in the adenohypophysis immediately after the operation, but a pronounced reduction in the number of the cells occurred during the first month after castration and lasted for 4 months thereafter. This pattern of change seems to agree well with the change in the pituitary LH content in the present series of experiments, and the administration of TP might favor the survival and/or restoration of pituitary cells in the shrew.

Although the feedback effect of androgens on LH secretion is generally considered to be entirely negative, there are several bodies of evidence suggesting the presence of positive effect in the rat (Swerdloff and Walsh, 1973; Verjans and Eik-Nes, 1977). Swerdloff and Walsh (1973) reported that the injection of low doses of TP (30 $\mu\text{g}/100\text{ g body weight} \times 5\text{ days}$) resulted in an elevation of the serum level of LH in chronically castrated male rats. Thus, the positive feedback possibility deserves consideration in explaining the increase in the LH content caused by TP treatment in the shrew 30 days after castration. The feedback mechanism between pituitary and gonadal functions in the shrew may differ considerably from that in other mammalian species, although a final conclusion cannot be drawn until the blood levels of the hormone are determined.

The decrease in the weight of accessory sex organs in the castrated male shrews was completely prevented by the TP replacement started immediately after the operation. However, the weight loss of the organs could not be fully restored to the level in intact animals by the same treatment when the injection of TP was begun at a later time post operation. The plasma testosterone concentration in castrated animals treated with 100 μg TP was about twice as high as that in intact animals. The dose of TP used in the present study, therefore, seemed to be enough to maintain the normal function of the accessory sex organs. A larger amount of the hormone or a longer period of treatment might be needed for restoration of the structure and the function of once degenerated organs as reported in the rat (Heller *et al.*, 1943). In the female shrew, ovariectomy and estrogen replacement from 3 months after the operation did not induce any change in weight or histology in the uterus and the vagina (Dryden and Anderson, 1977). We also observed the lack of uterine response to estrogen (1.0 μg of estradiol $\times 15\text{ days}$) in the ovariectomized shrew, though

the vaginal epithelium showed extensive proliferation (Tokuyama, unpublished). Such an unusual features in the response to gonadal steroid observed in the female uterus are not seen in the case of the male accessory sex organs.

Orchidectomy also decreased the weight of the musk gland and the treatment with TP restored weight considerably. Dryden and Conaway (1967) reported that the decrease in weight of the musk gland in the castrated shrew could be recovered by treatment with estradiol and progesterone as well as TP. Although the musk gland may not be a major scent gland in the species (Dryden and Conaway, 1967), the relation between the function of this gland and gonadal steroids might be studied.

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